# Panviral Lipid Remodeling for Replication

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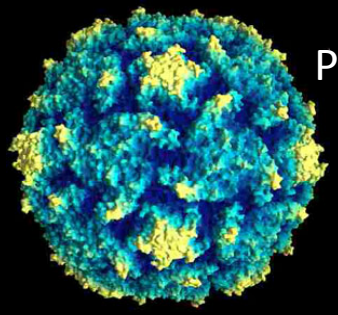
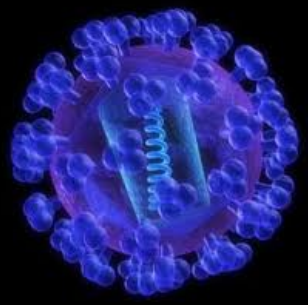
Laboratory of Host-Pathogen Dynamics
NHLBI

# SINGLE POSITIVE STRAND RNA VIRUSES

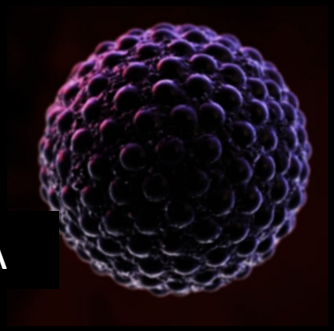


Poliovirus

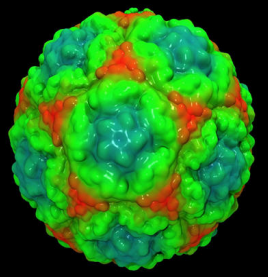
Hepatitis C



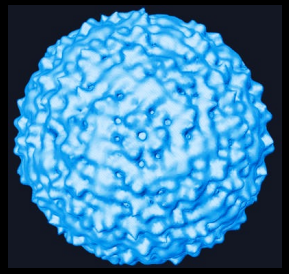
Coxsackievirus



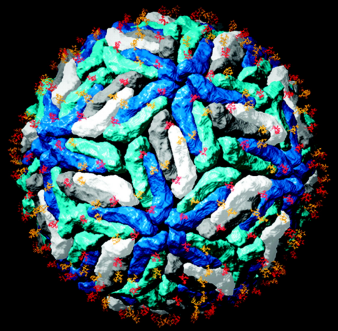
Hepatitis A



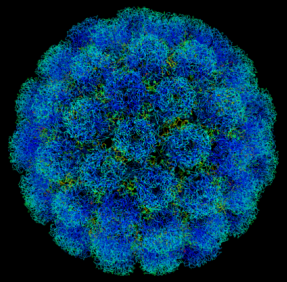
Rhinovirus



West Nile virus



Dengue virus



Enterovirus 71

# RNA viruses (relative to DNA viruses):

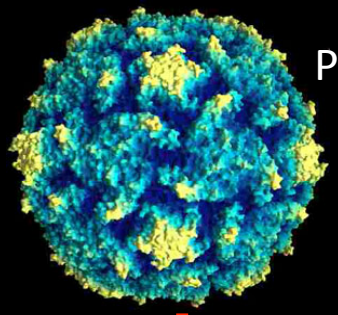
- Small genome size: multiple distinct enzymatic activities encoded by polytopic proteins.
- High mutation rate: impaired error correction.
- Large variation in genomic sequence within a single host cell
- Evolution and selection in short time scales.

Rapid emergence of resistance to therapeutics

Alternative strategy to
kill RNA viruses

Target host factors
that RNA viruses need for
replication

# SINGLE POSITIVE STRAND RNA VIRUSES

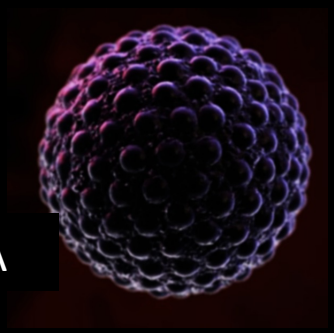


Poliovirus

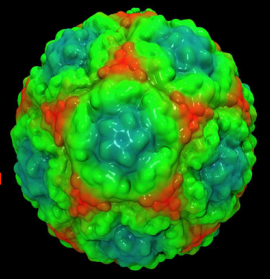
Hepatitis C

Coxsackievirus

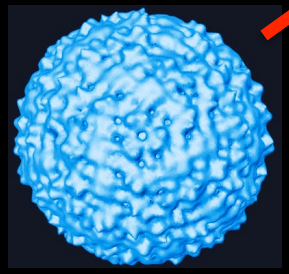
**Membrane platforms for replication and assembly**



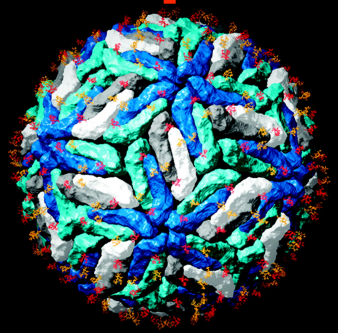
Hepatitis A



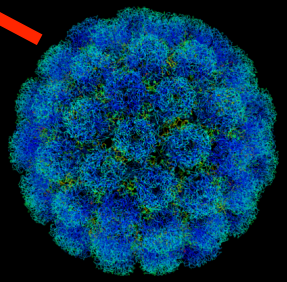
Rhinovirus



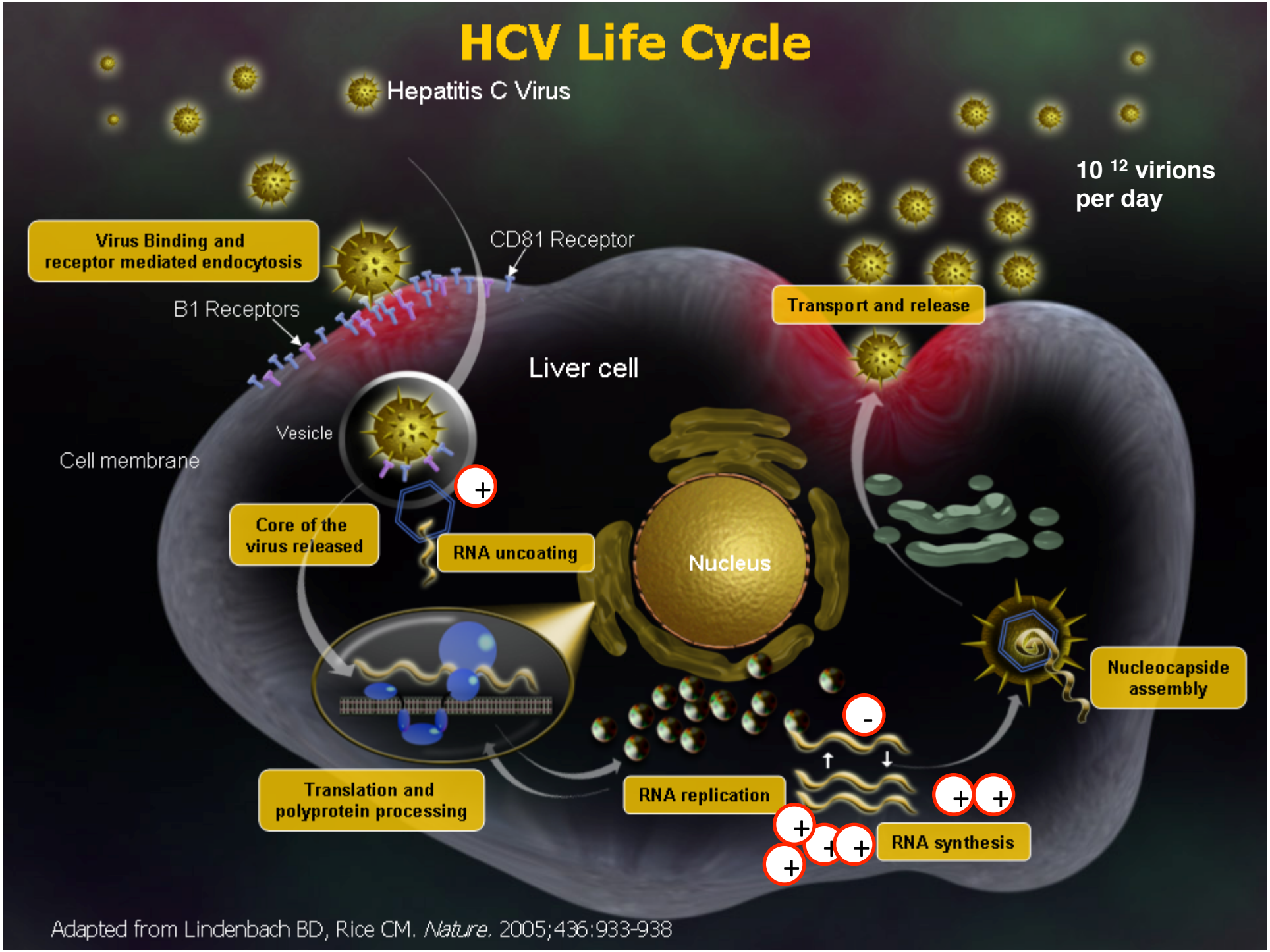
West Nile virus



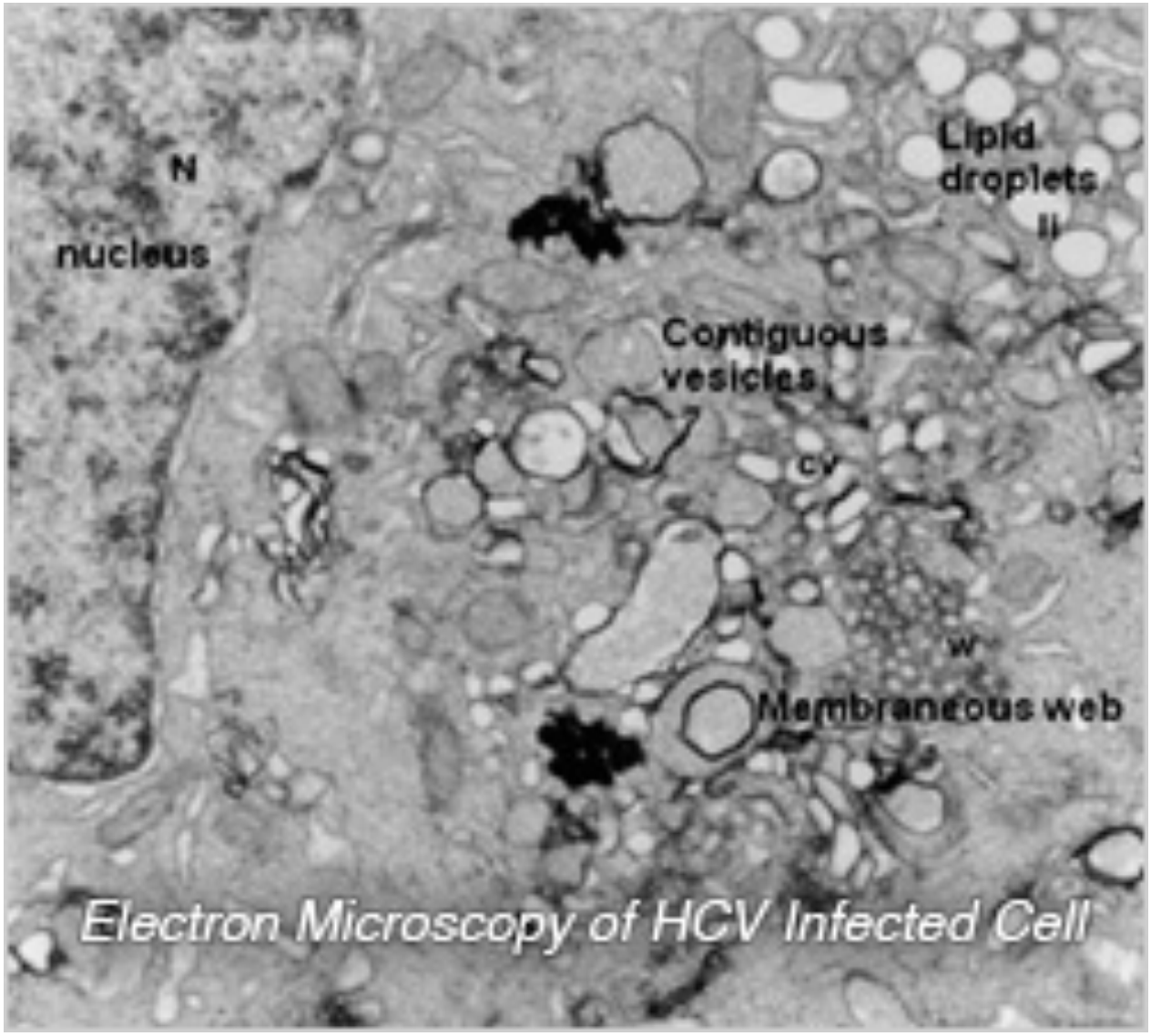
Dengue virus



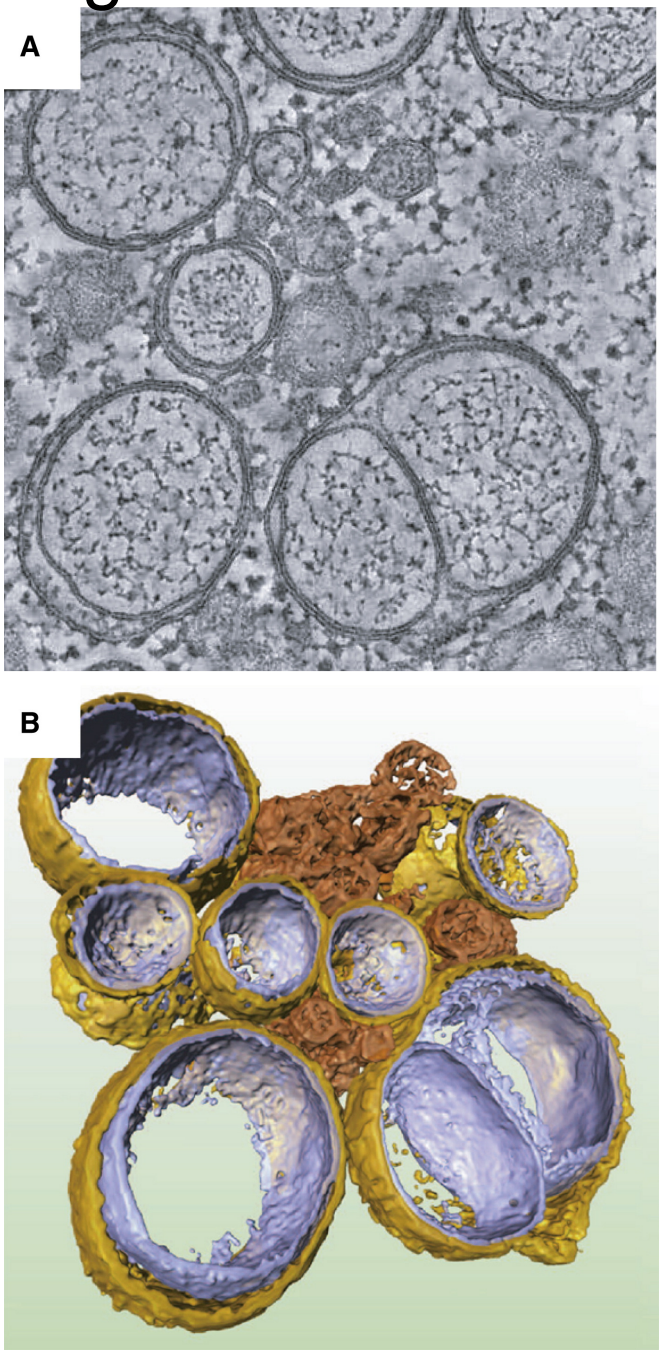
Enterovirus 71


HCV Life Cycle
Hepatitis C Virus
10 12 virions per day
Virus Binding and receptor mediated endocytosis
CD81 Receptor
B1 Receptors
Liver cell
Transport and release
Vesicle
Cell membrane
Core of the virus released
RNA uncoating
Nucleus
Nucleocapside assembly
Translation and polyprotein processing
RNA replication
RNA synthesis
Adapted from Lindenbach BD, Rice CM. Nature. 2005;436:933-938

# HCV Replication organelle
# “membranous web”

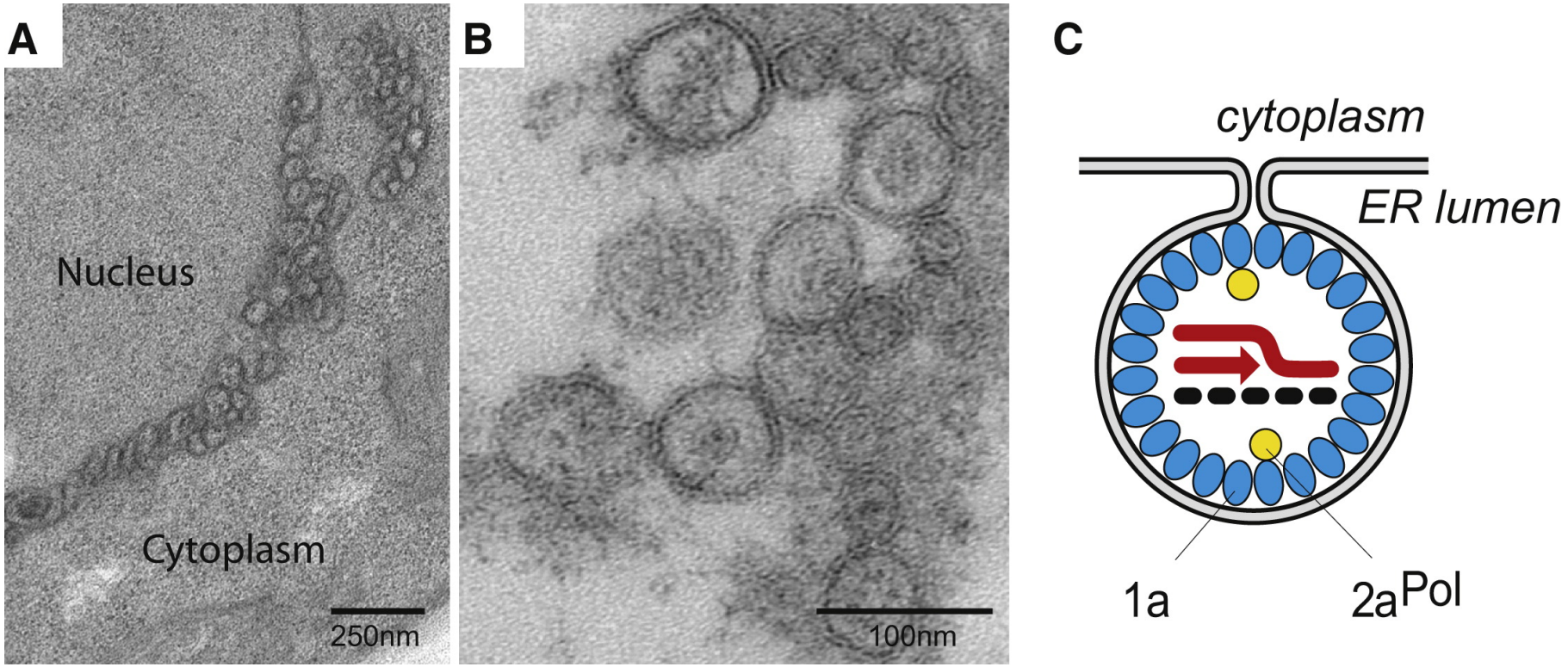


# SARS replication organelles



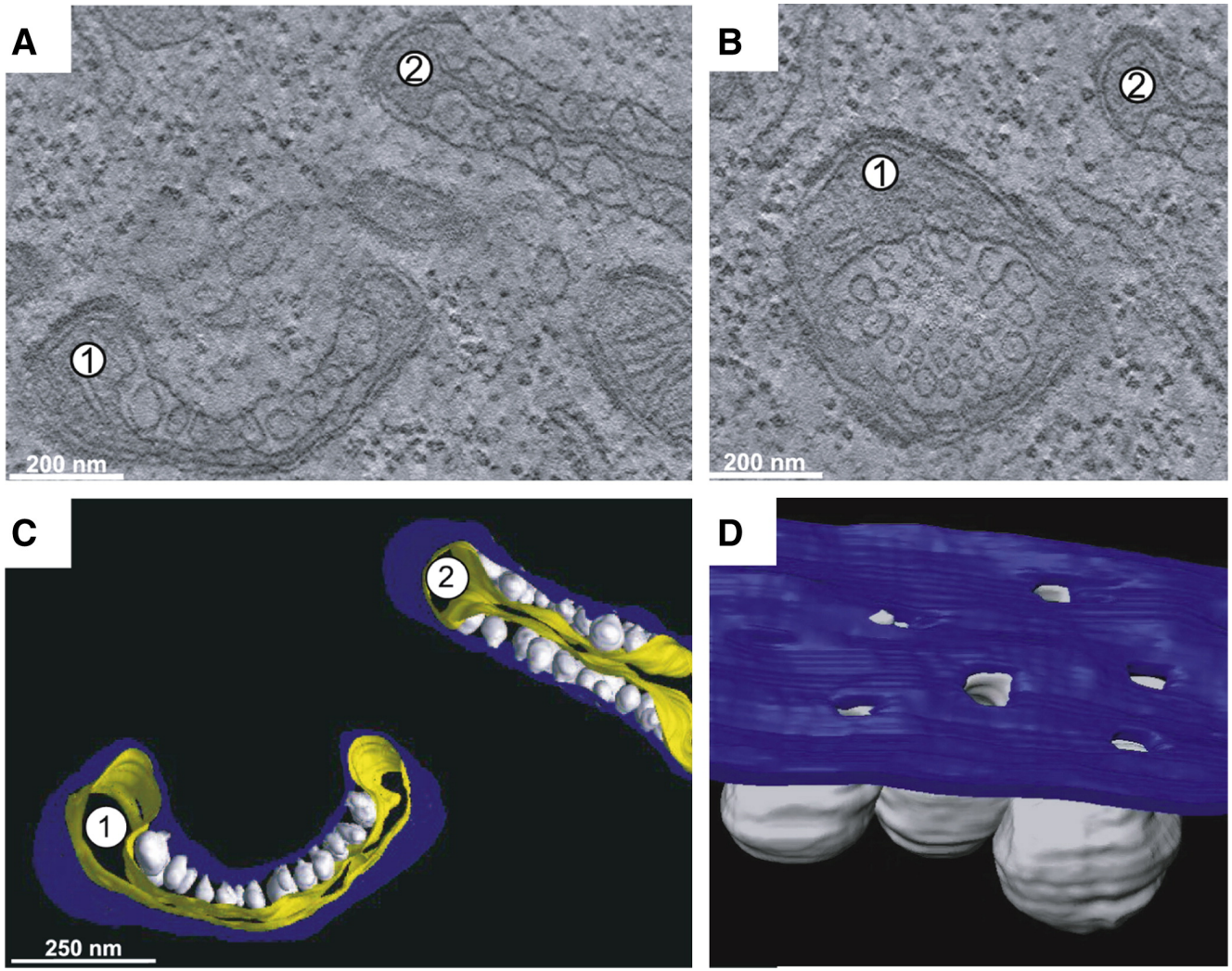

Ahlquist et al., Cell 2010

# Bromomosaic virus replication organelles



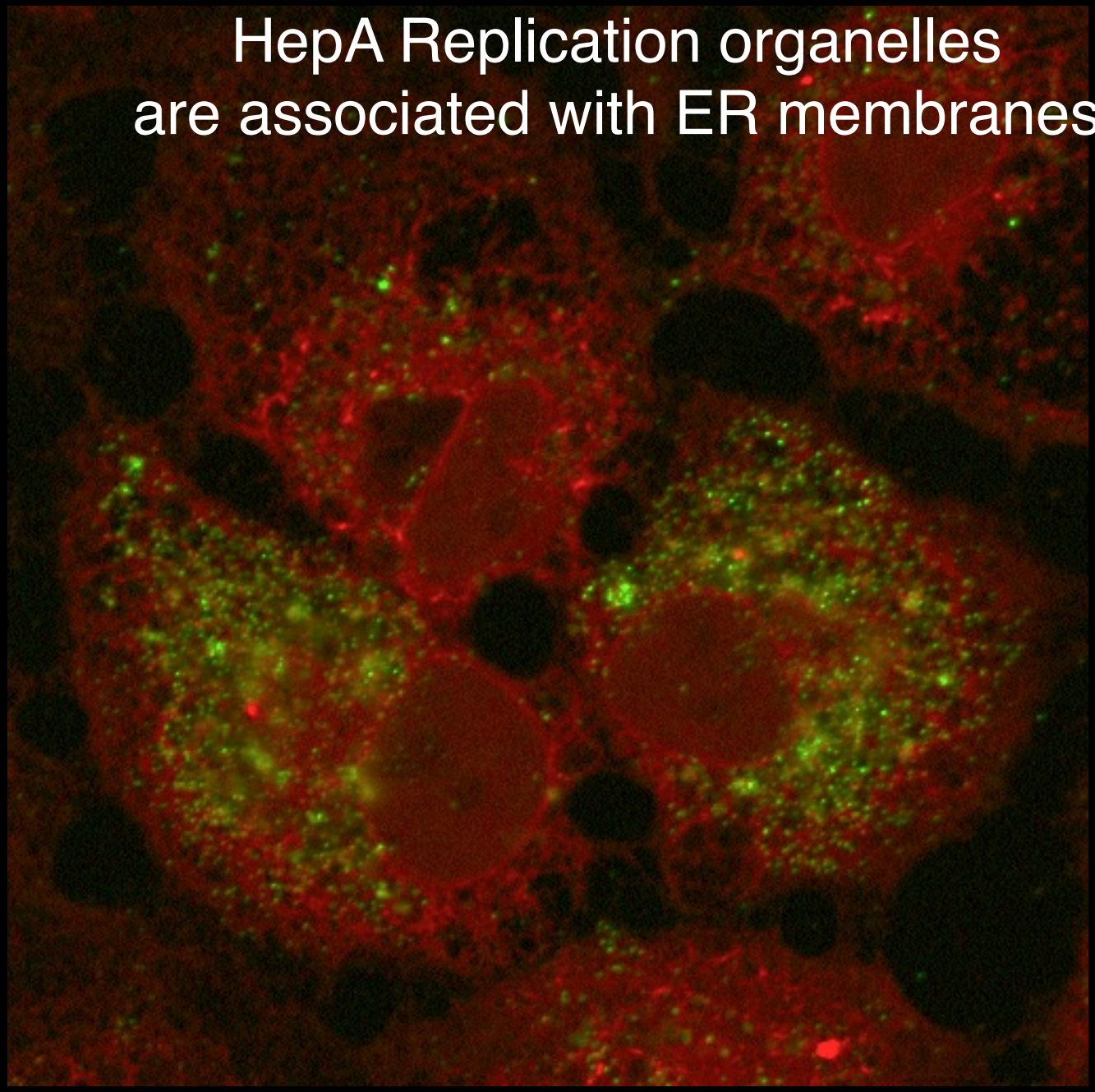

Ahlquist et al., Cell 2010

# Flockhouse virus replication organelles

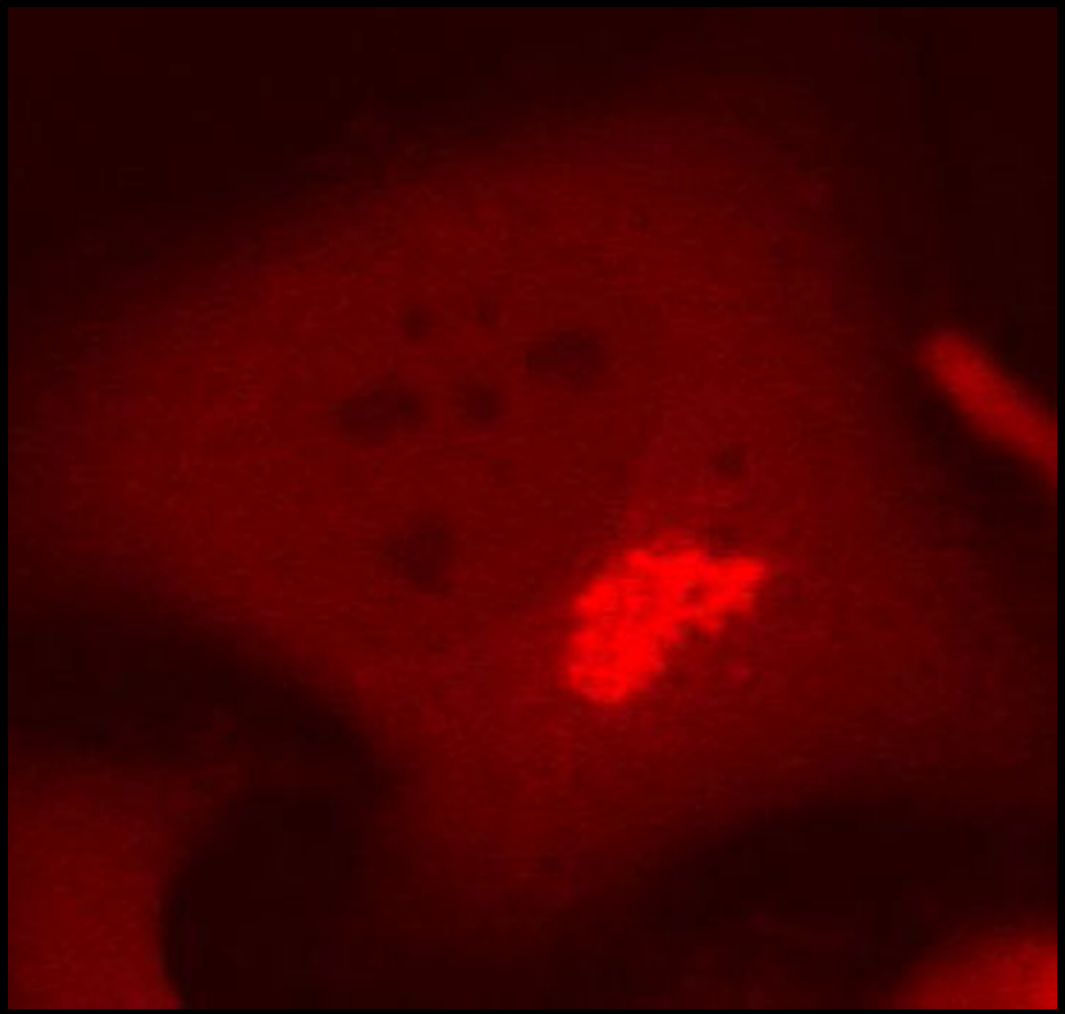



Ahlquist et al., Cell 2010

# HepA Replication organelles are associated with ER membranes



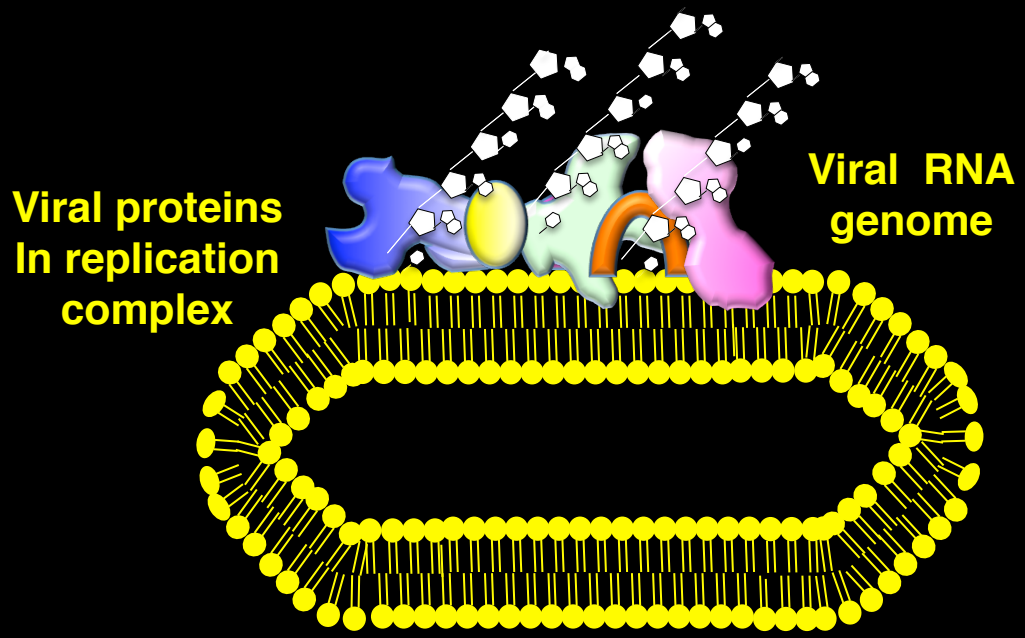
# Formation of the Viral replication factories in living cells



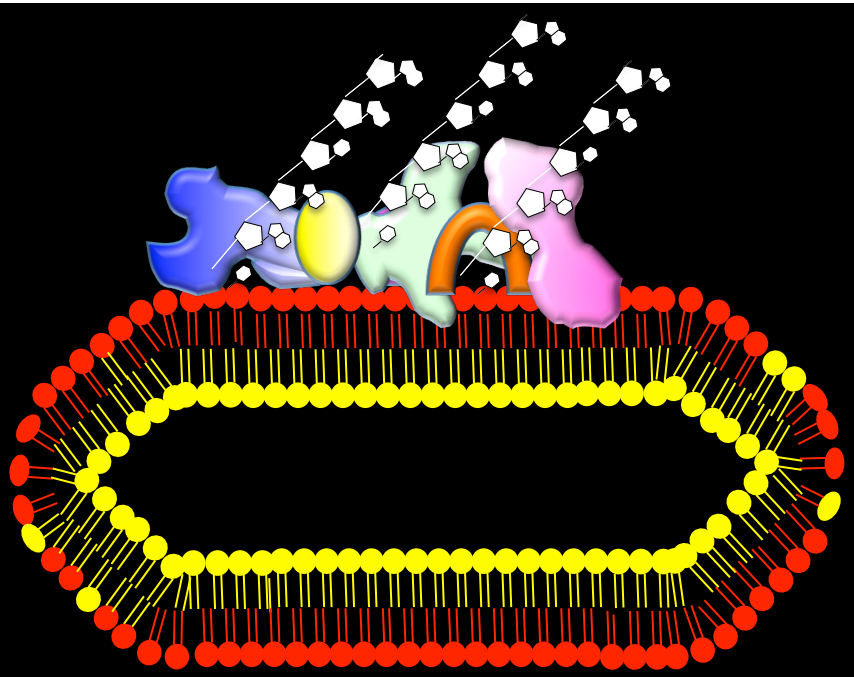
# Why Replicate on Membranes ?

**Early in infection viral proteins are low in abundance-**

# Viral Replication on membranes




**Affinity of viral proteins for membranes limits their diffusion, thus decreasing the entropy loss, which may facilitate assembly of proteins into a replication complex.**



**What kind of lipids do viral proteins like to bind to?**

**Additional novel roles for lipids in viral replication?**

**Are there specific lipids that multiple different RNA viruses require for replication?**

# PI4P lipid enriched Replication membrane platforms are generated

Poliovirus

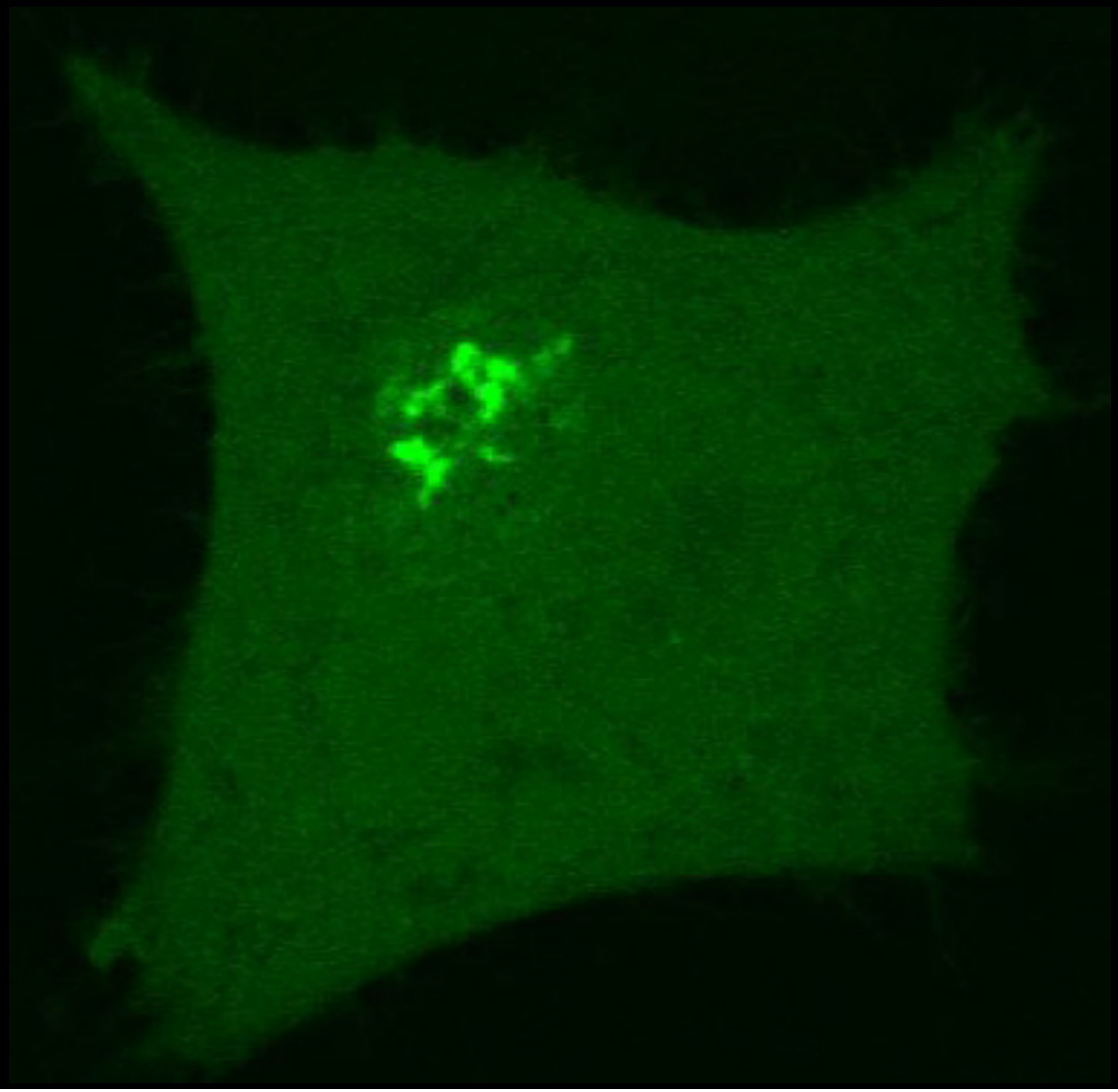
Coxsackivirus

Rhinovirus

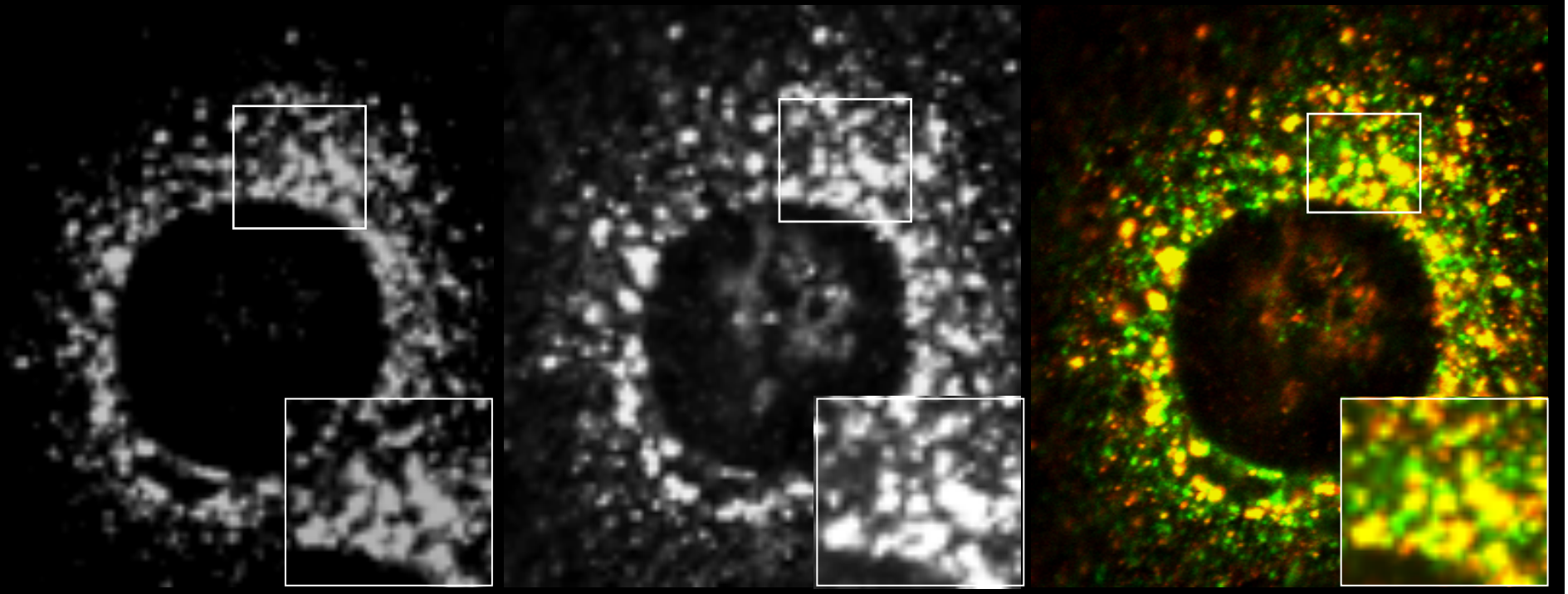
Echovirus

Aichivirus

Enterovirus 71



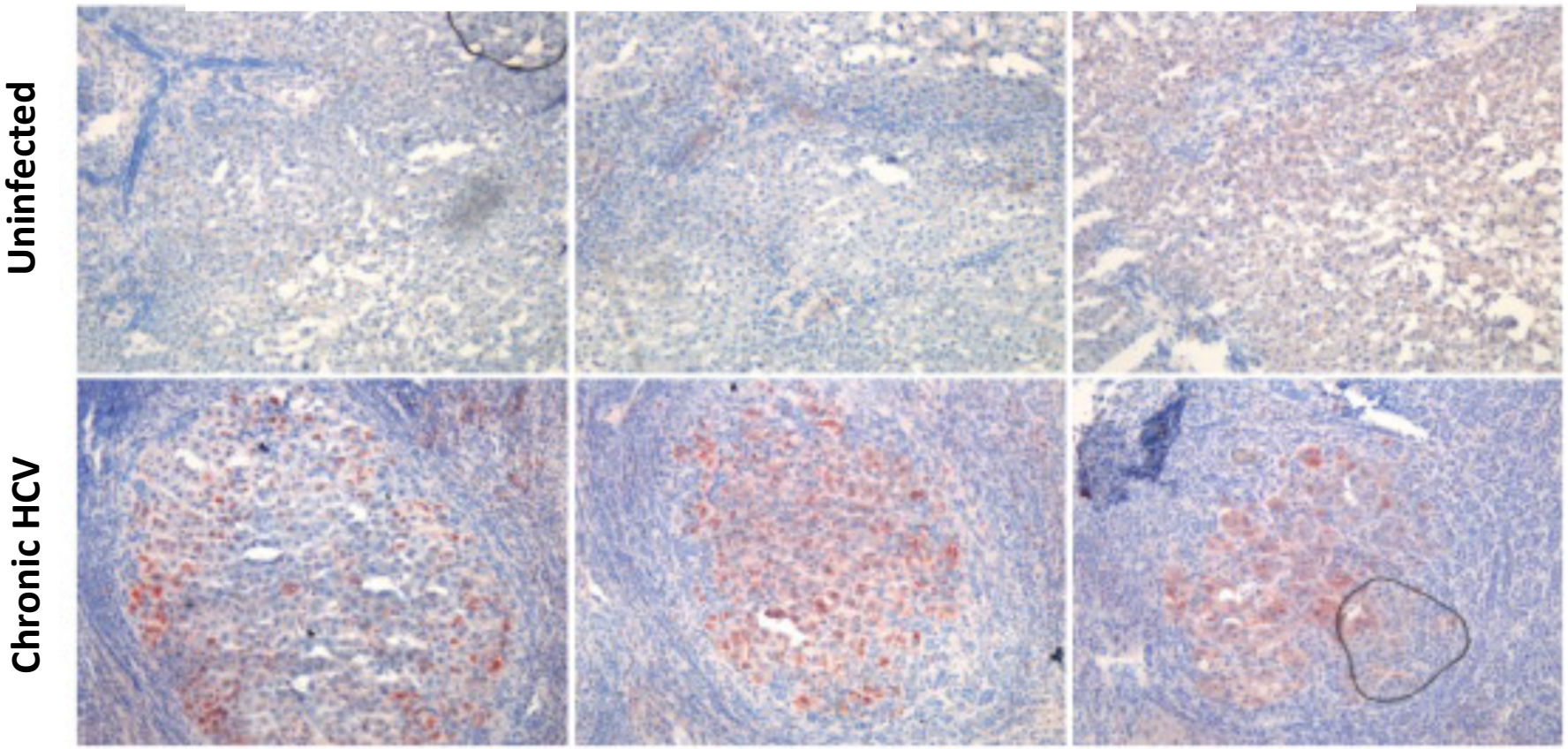
# HCV replication organelles are also highly enriched in PI4P lipids



**NS5A** **PI4P** **PI4P NS5A**

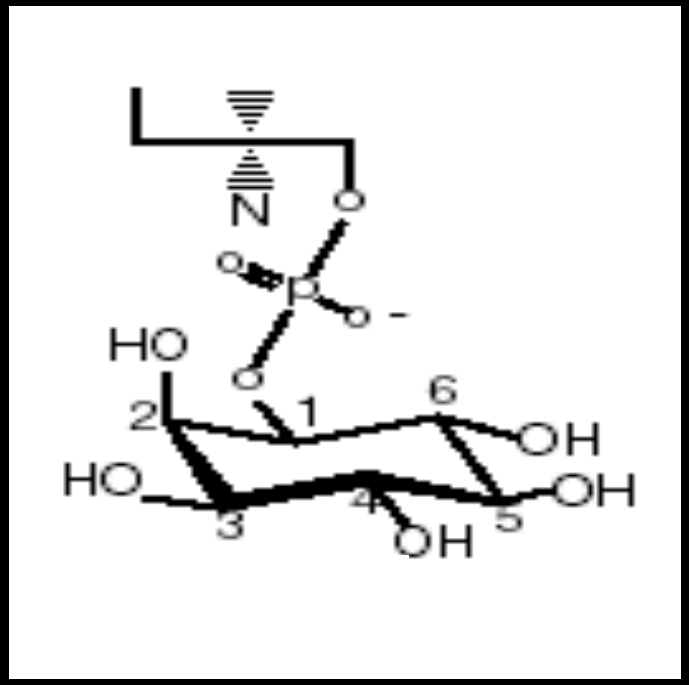
Hsu et al., Cell 2010

# Livers of patients with chronic HCV are highly enriched in PI4P lipids

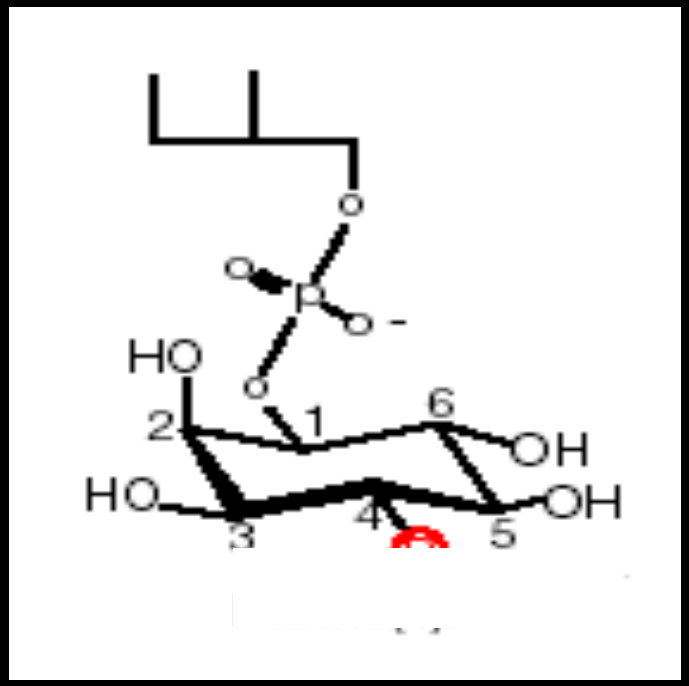



Reiss et al., 2011 Cell Host and Microbe

# Phosphotidylinositol 4 kinases generate PI4P lipids

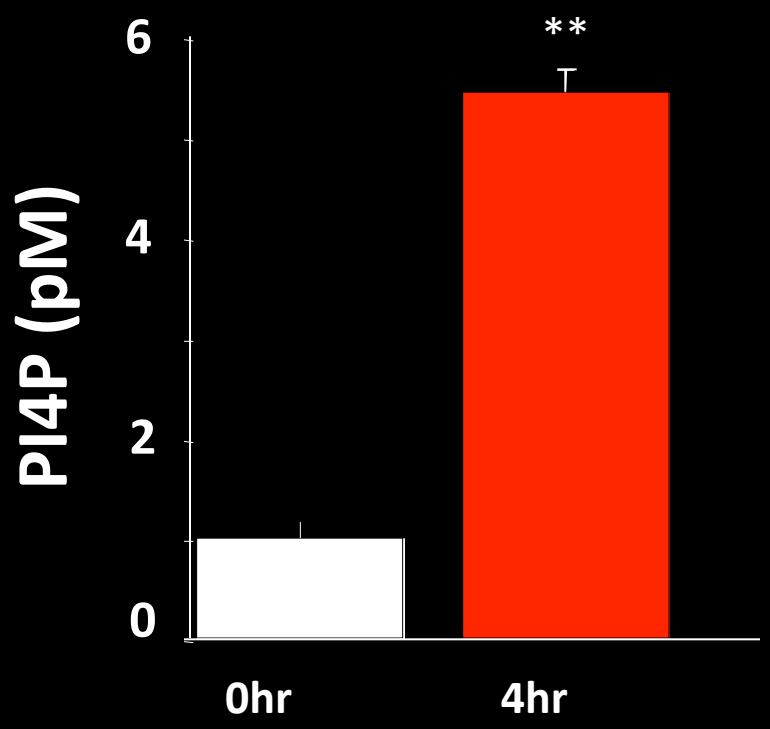



Phosphatidylinositide (PI)

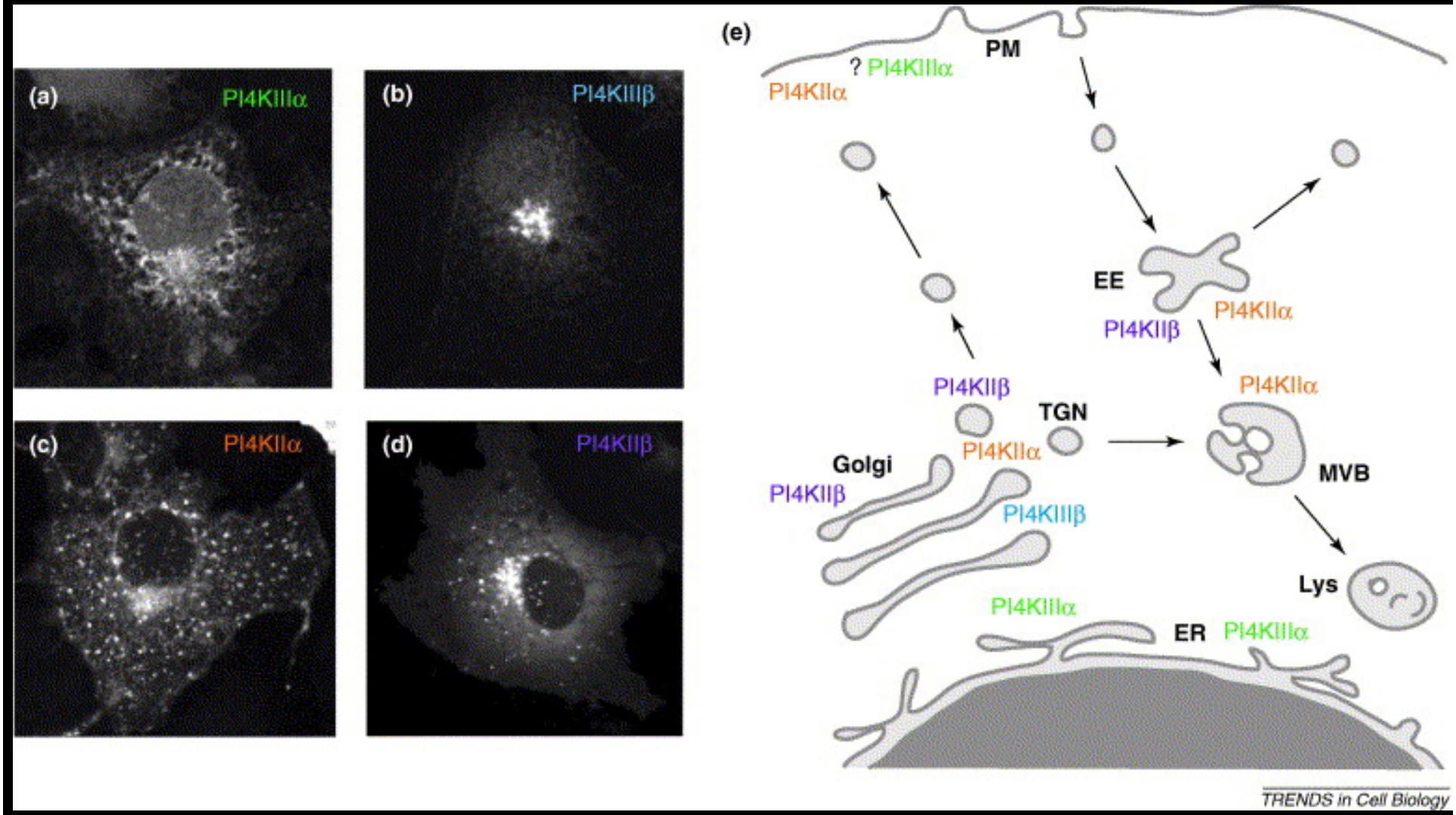



Phosphatidylinositide
4-phosphate (PI4P)

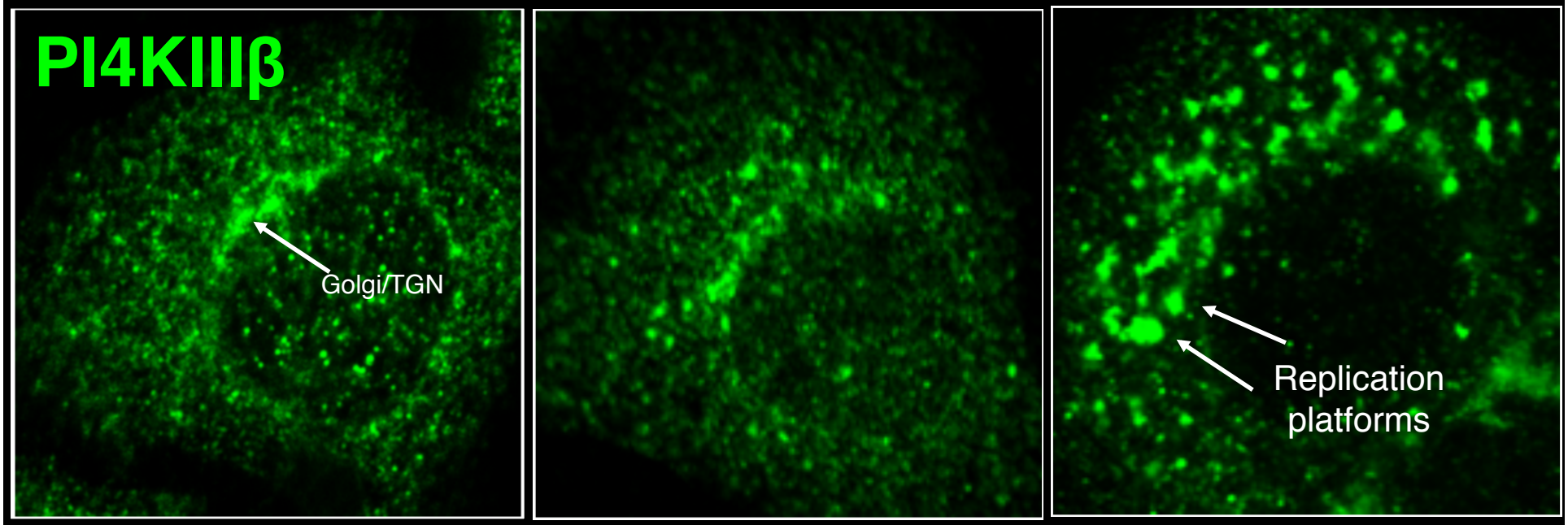
# PI4P lipid levels increase > 5 fold during infections



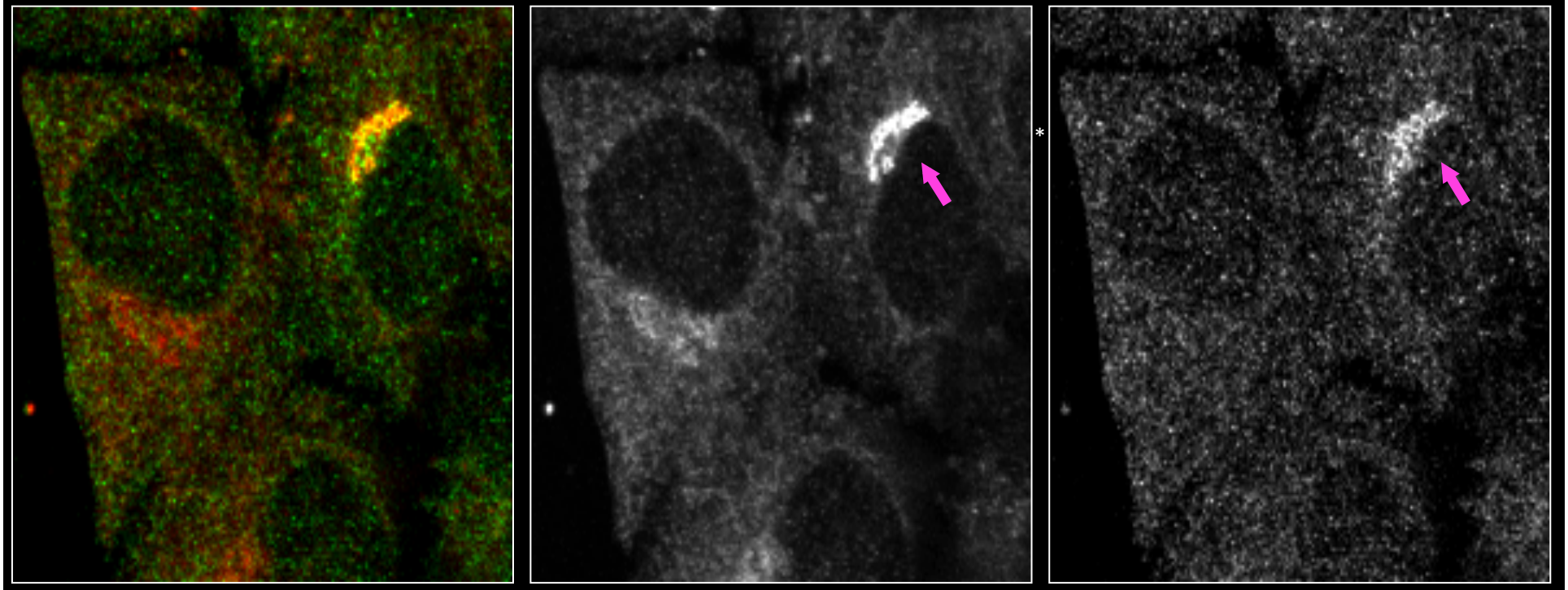

Hsu et al., Cell 2010


(a)
PI4KIIIα
(b)
PI4KIIIβ
(c)
PI4KIIα
(d)
PI4KIIβ
(e)
PM
? PI4KIIIα
PI4KIIα
EE
PI4KIIα
PI4KIIβ
PI4KIIβ
PI4KIIα
TGN
PI4KIIα
Golgi
PI4KIIβ
MVB
PI4KIIIβ
Lys
PI4KIIIα
ER
PI4KIIIα
TRENDS in Cell Biology

# RNA viruses hijack a specific host lipid kinase, **PI4KIIIβ** to generate PI4P enriched replication platforms



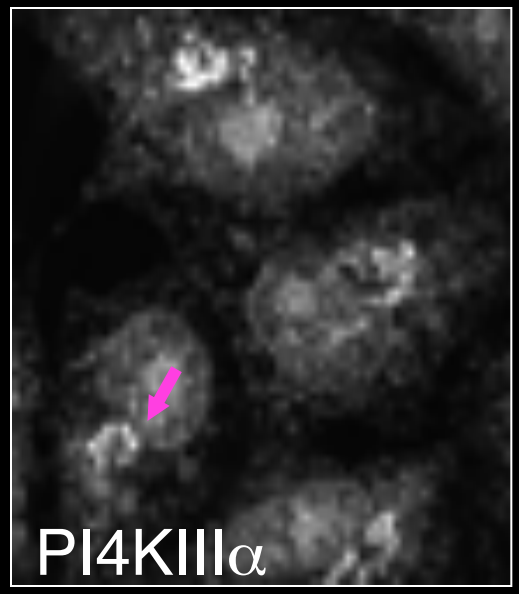
# 3A expression enhances PI4KIIIβ recruitment to membranes

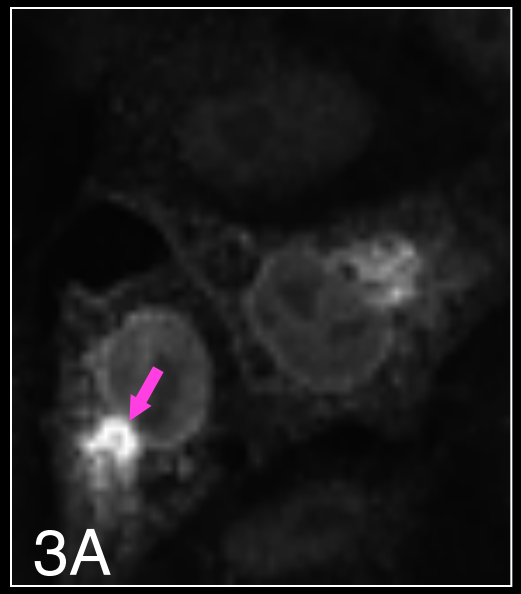


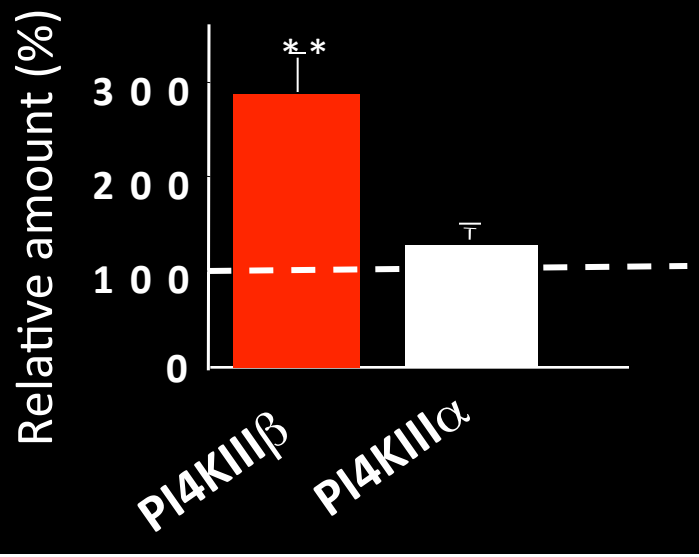
PI4KIIIβ 3A PI4KIIIβ 3A

Hsu et al., Cell 2010

# 3A effect on PI4KIIIβ recruitment is highly selective

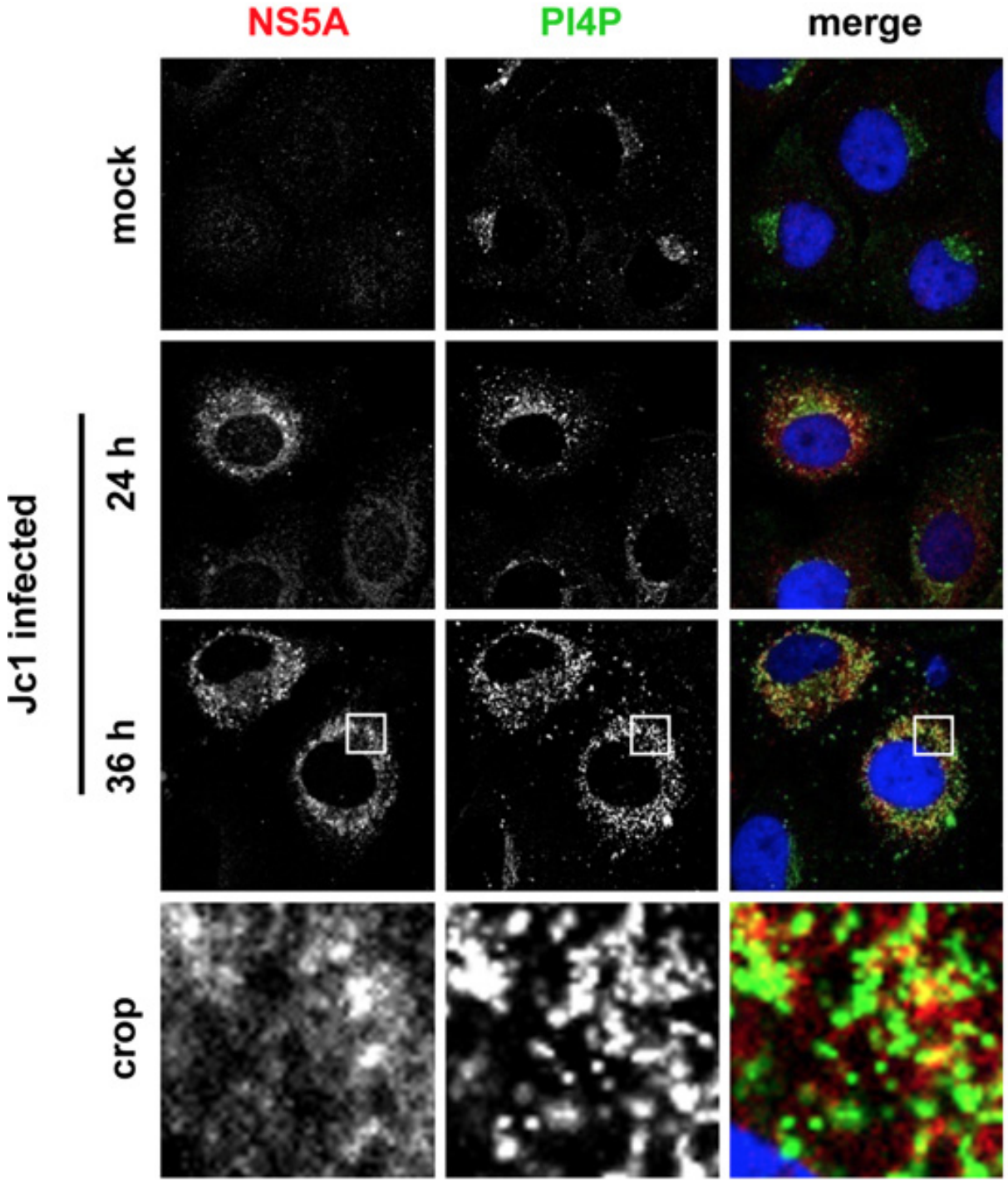



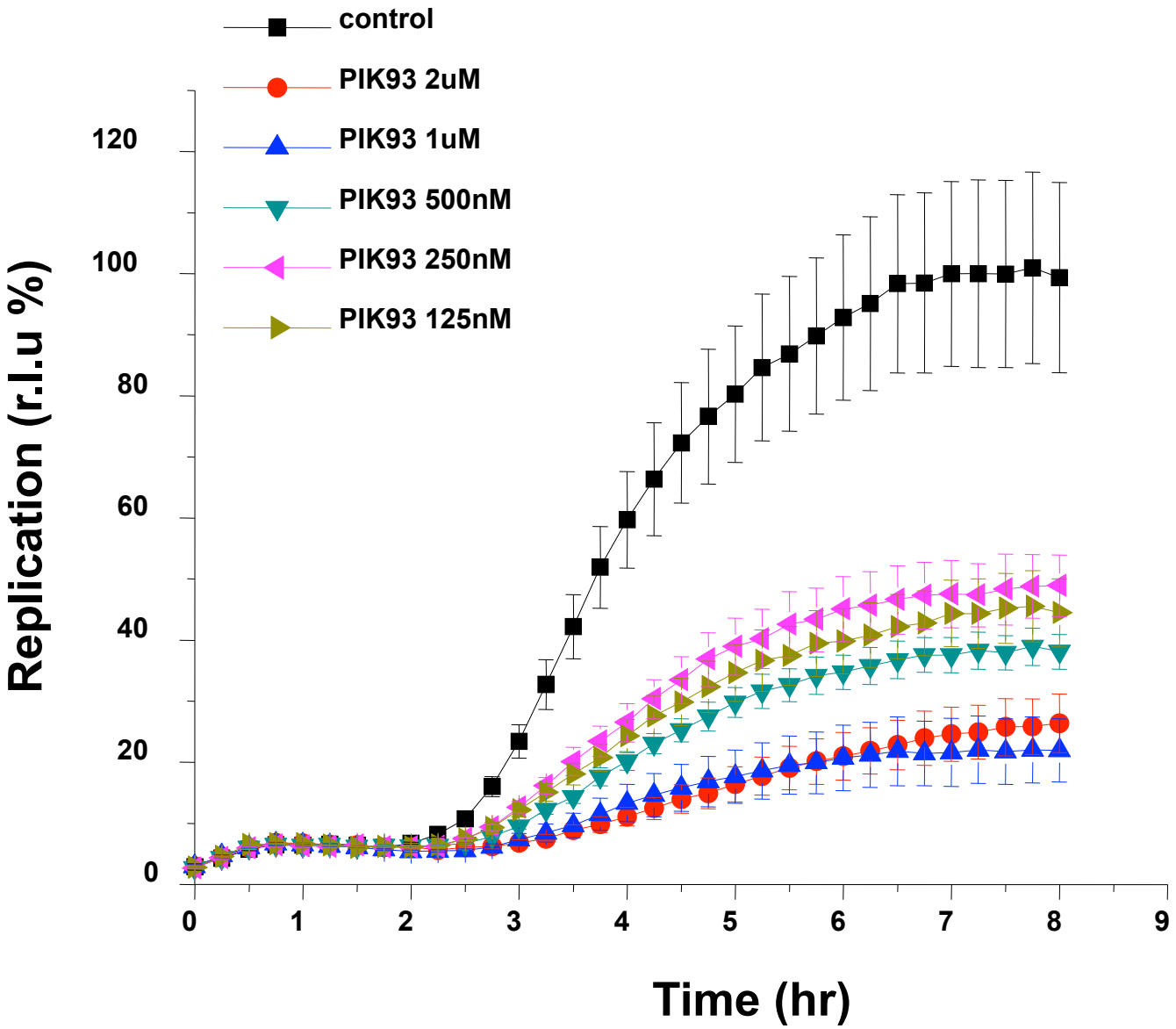

Baseline membrane levels

Hsu et al., Cell 2010



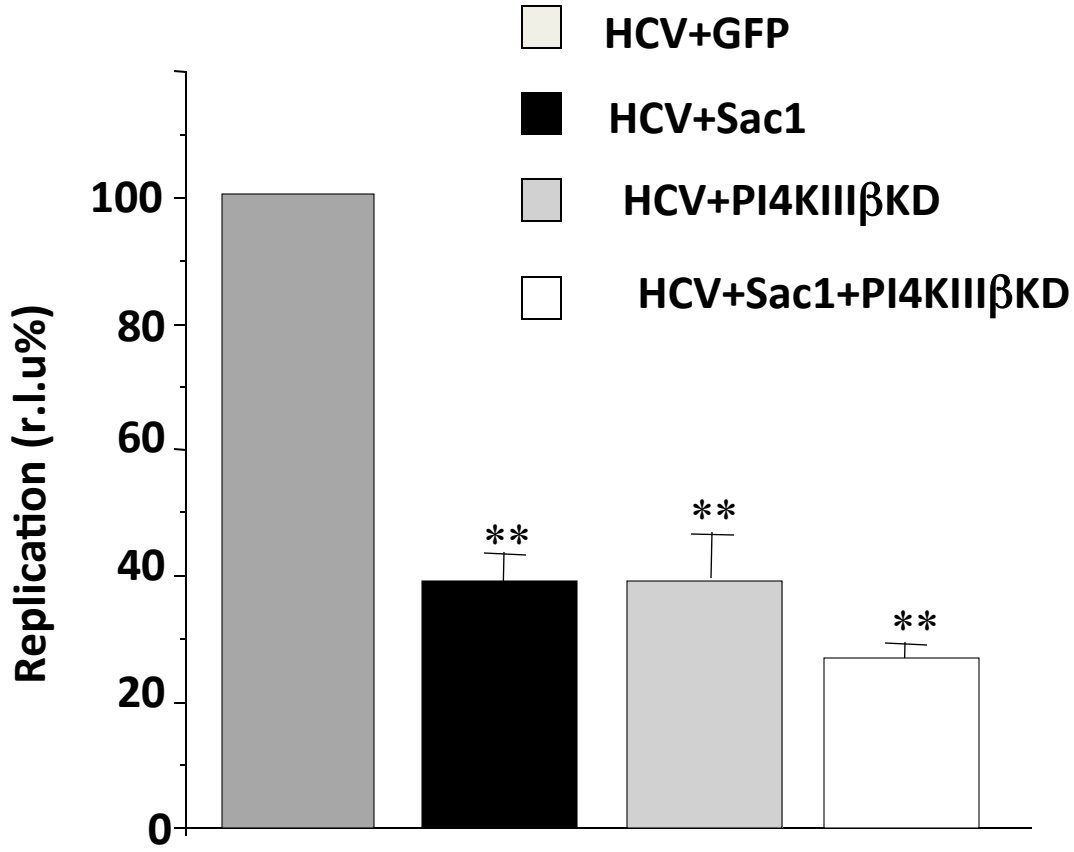

Reiss et al., 2011
Cell Host and Microbe

# PIK93 effectively inhibits Enteroviral Replication




Hsu et al., Cell 2010

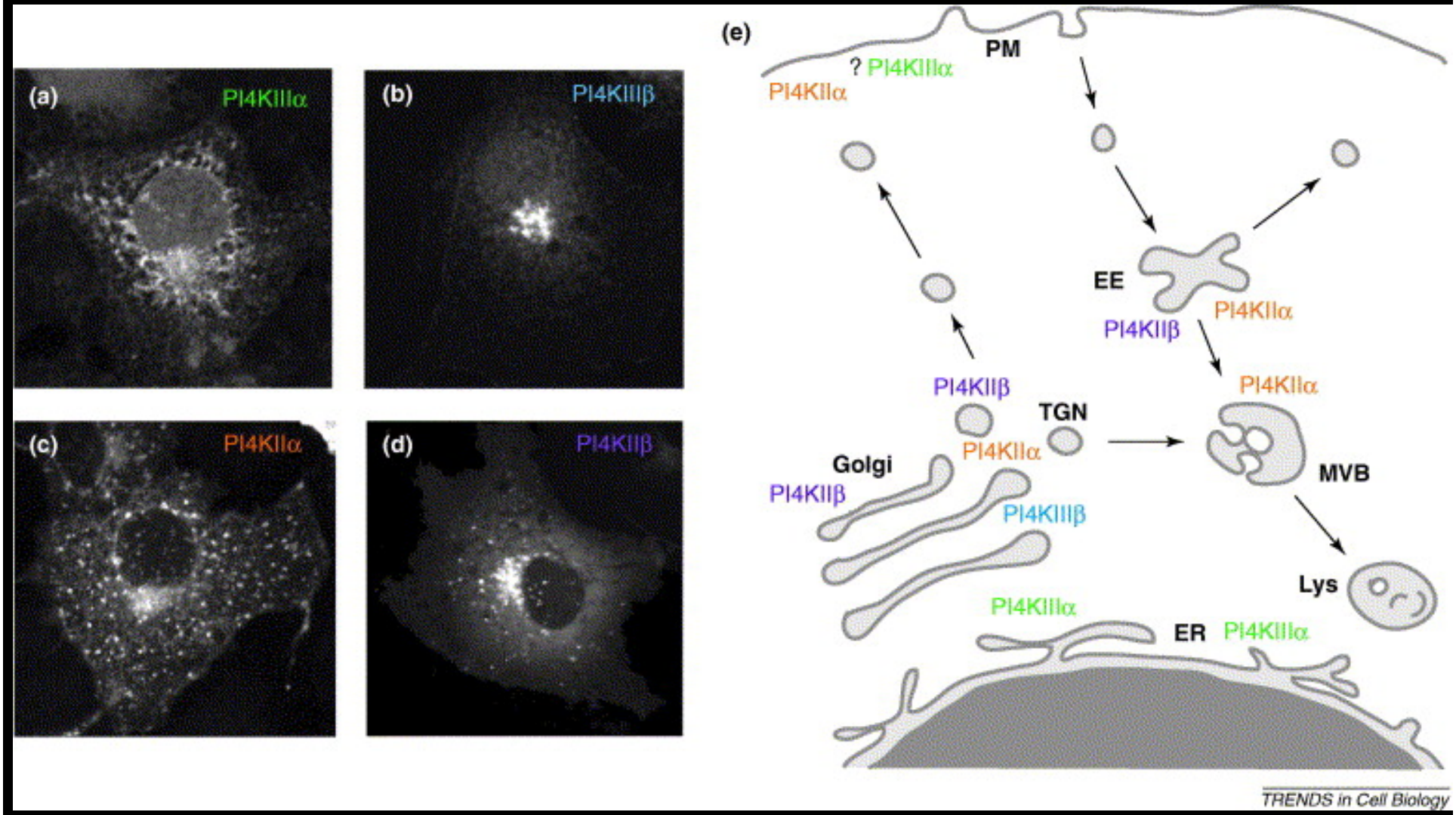
# Inhibiting PI4P lipid production inhibits HCV RNA replication




HCV J6/JFH (p7-Rluc2A) replicon

Hsu et al., Cell 2010

| Treatment | Cell Viability (% mean of Control cells +/-S.E.M) |
|---|---|
| Type III PI4K β siRNA (4 days) | 115.0 +/- 4.5 |
| Type III PI4K β kinase-dead (4 days) | 105.7 +/- 10.6 |
| PIK 93 (up to 5μM for 3 day treatment) | 114.4 +/- 10 |


(a)
PI4KIIIα
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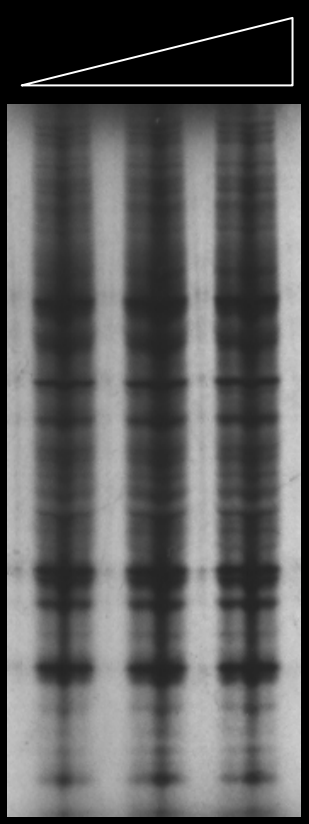
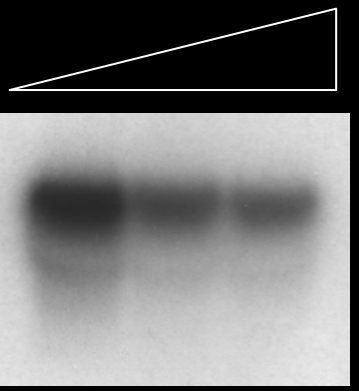
PI4KIIβ
PI4KIIα
TGN
PI4KIIα
Golgi
PI4KIIβ
MVB
PI4KIIIβ
Lys
PI4KIIIα
ER
PI4KIIIα
TRENDS in Cell Biology

# PI4P lipids specifically regulate RNA synthesis and do not impact translation



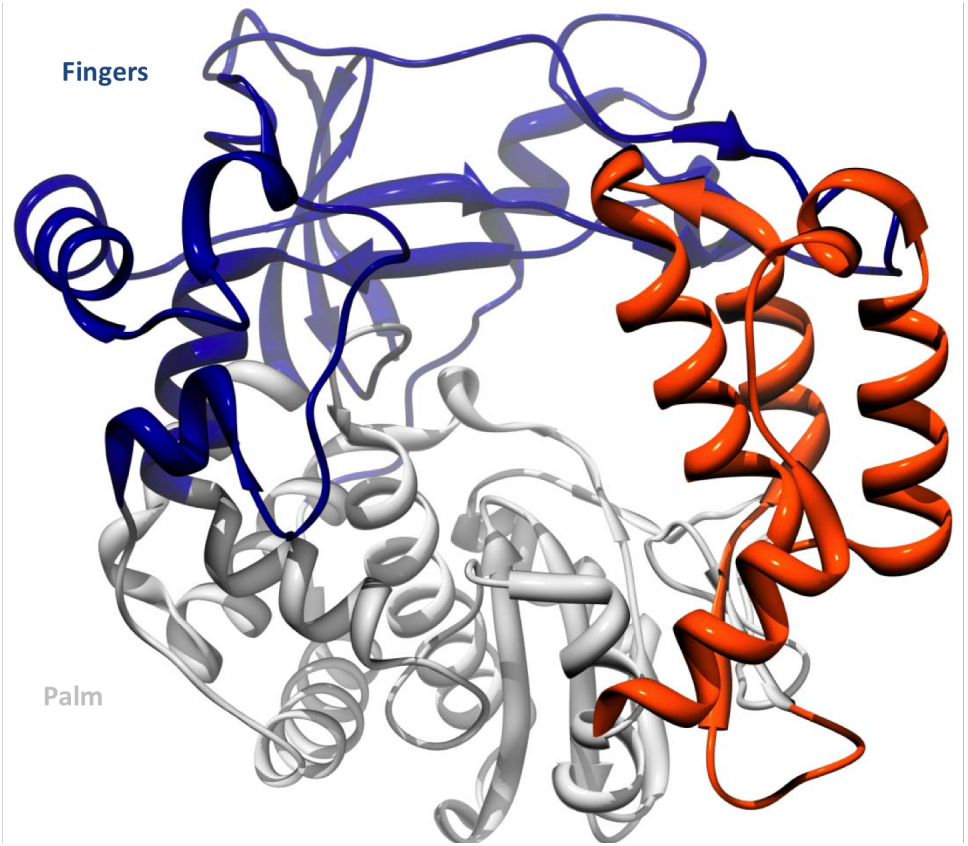

Viral proteins




Cell-free assays where RNA translation and synthesis can be uncoupled and impact of drugs on each can be tested.

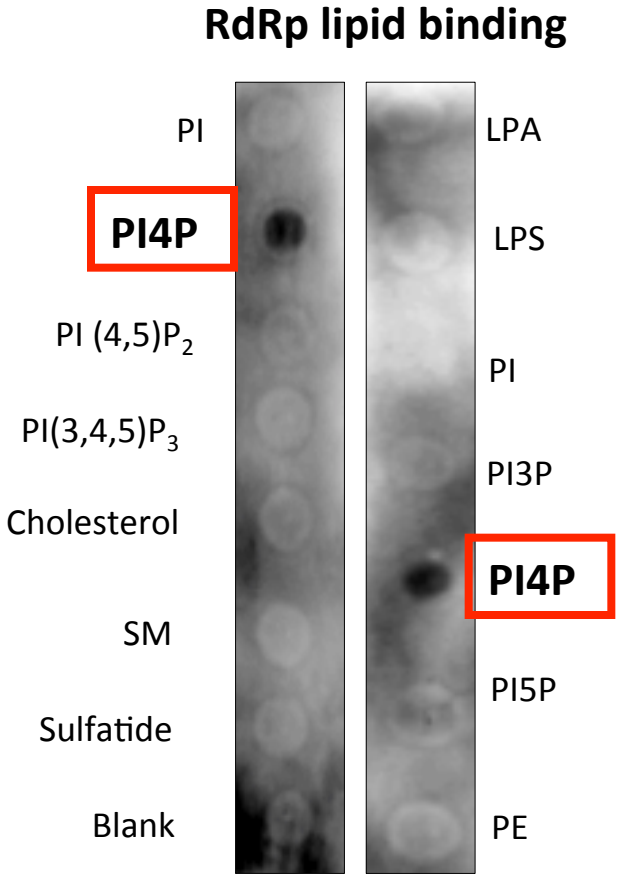
Hsu et al., Cell 2010

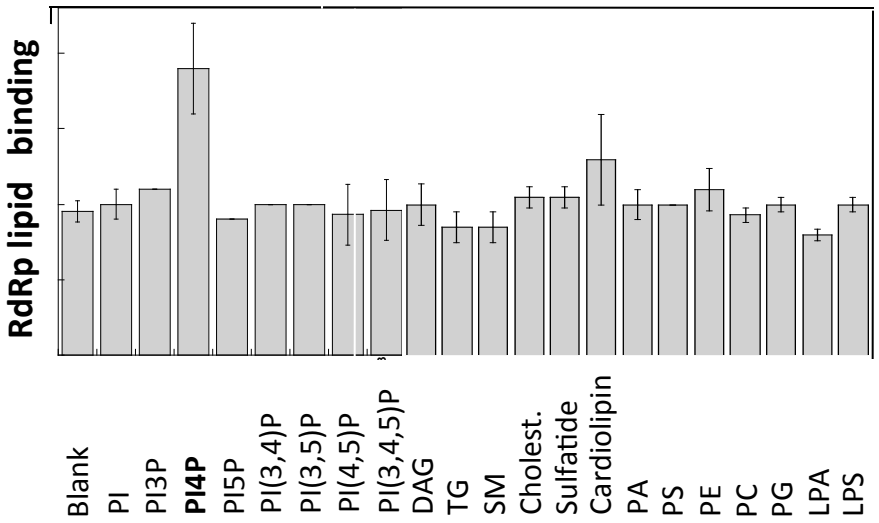
# Enteroviral RNA- dependent RNA polymerase (RdRp)




-soluble protein
-highly conserved structure
-activities include Vpg Uridylation, RNA polymerization

# Enteroviral RNA Polymerases specifically and preferentially bind PI4P lipids



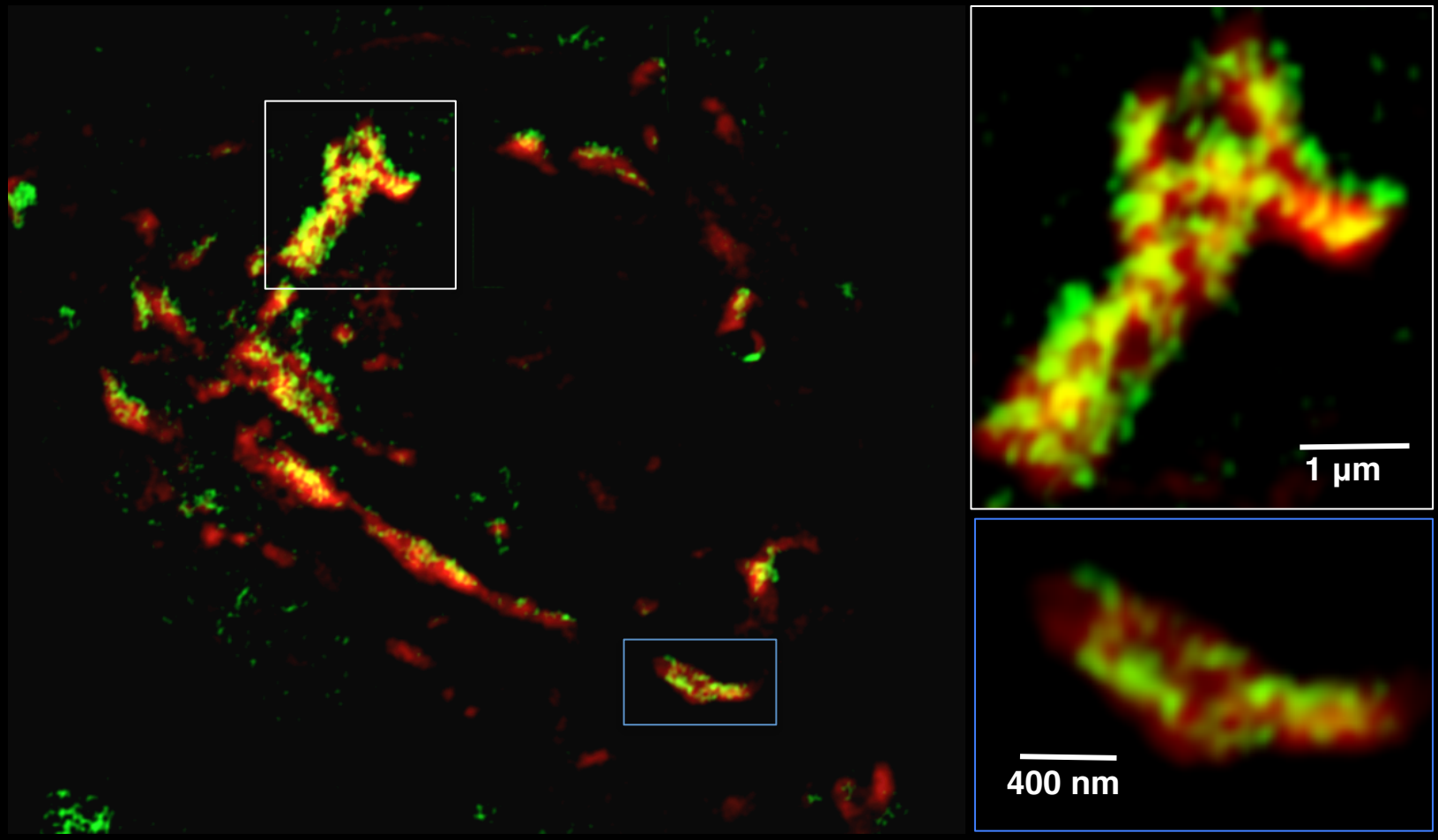

Hsu et al., Cell 2010

# Future Studies in Lab

- Other Lipids?

- Other lipid harnessing pathways?

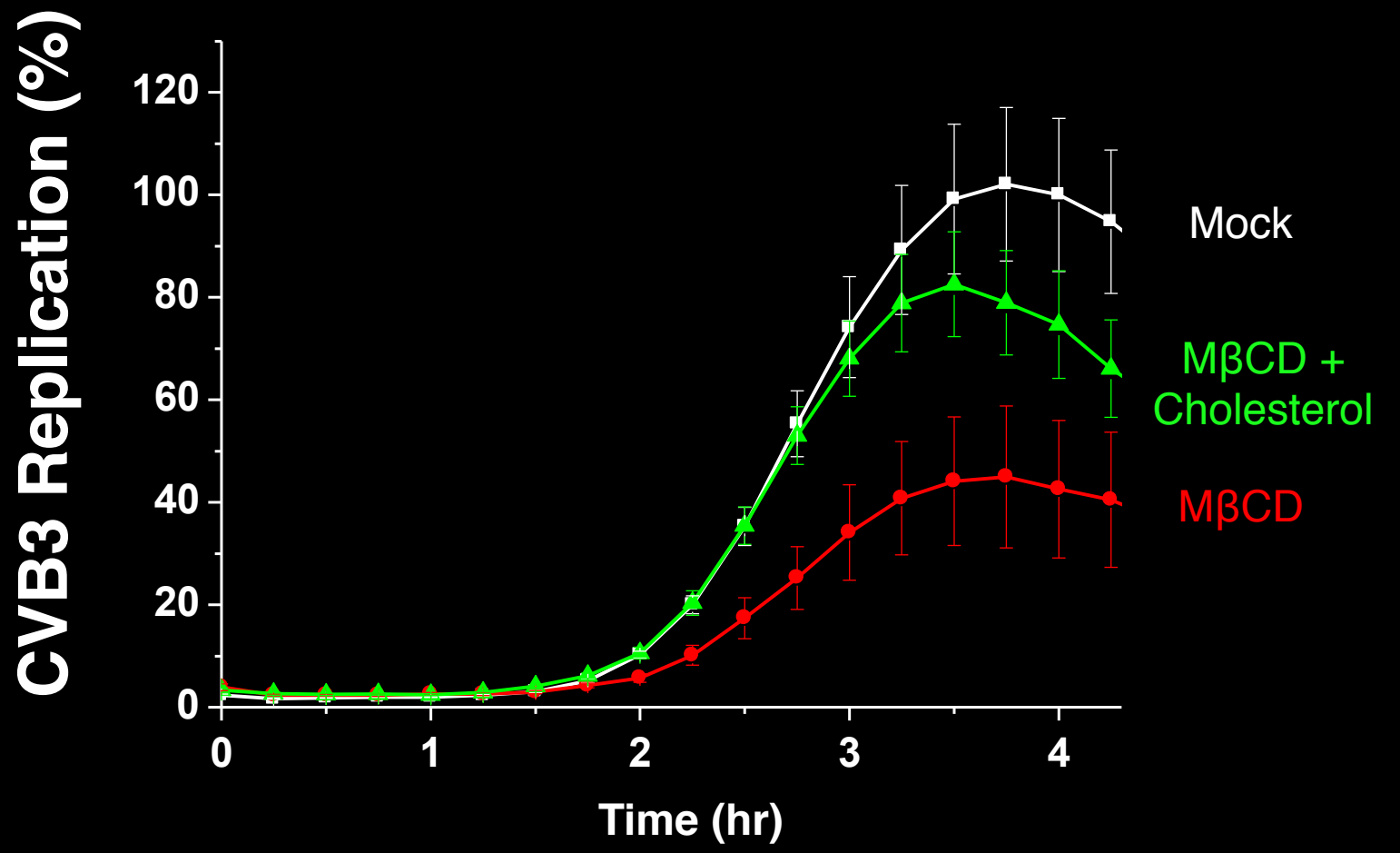
# Replication platforms are also enriched in cholesterol




**PI4P lipids**
**Phosphatidylinositol 4-phosphate**

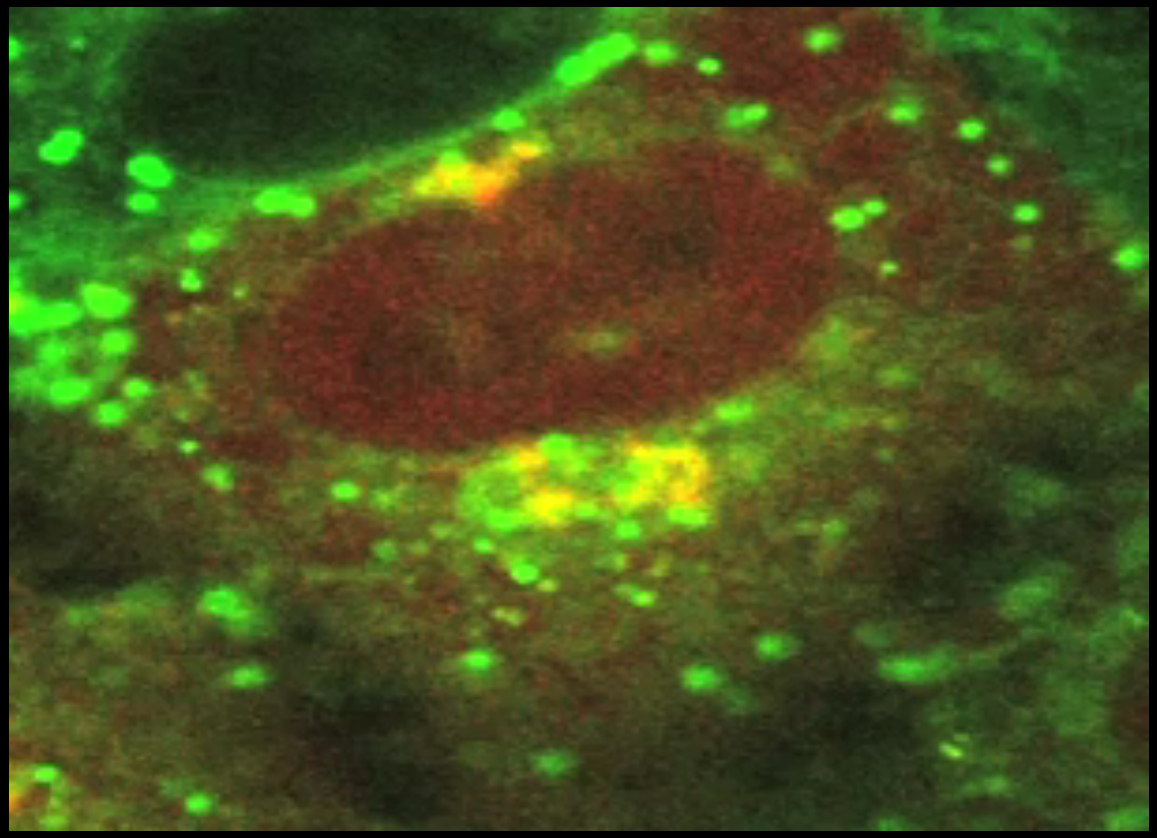
**cholesterol**

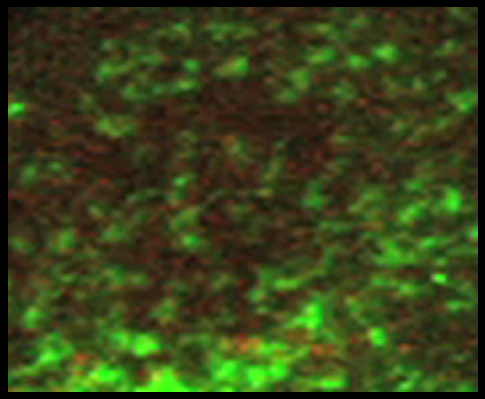
# Cholesterol regulates enteroviral replication




Ilnytska et al., Cell Host and Microbe 2013

# Plasma membrane free cholesterol pools are trafficked to replication organelles





**FAPP1-mRFP (replication organelles)**
**BODIPY-Cholesterol**

# CONCLUSIONS

- Multiple different viruses rely on specific lipids to regulate replication. Thus lipids may be good panviral therapeutic targets.

- Targeting lipids may have potentially limited negative impact on host functions since:

  Host may need fewer quantities of a specific lipid than the viruses does.

  Host may have multiple different mechanisms to generate a specific lipid; whereas viruses evolve to exploit one mechanism. For example: hijacking PI4KIIIβ.

- Association of viral proteins with specific lipids can facilitate replication complex assembly and enzymatic reactions.